THE SIGNIFICANCE OF PLEUROPNEUMONIA-LIKE OR 'L' ORGANISMS IN NON-GONOCOCCAL URETHRITIS, REITER'S DISEASE, AND ABACTERIAL PYURIA

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The first member of the group of pleuropneumonia organisms was isolated by Nocard and Roux in 1898 from cases of contagious pleuropneumonia of cattle, a disease in which the essential pathological lesions are gross pulmonary œdema, pleural exudate, and arthritis. A similar organism was found by Bridré and Donatien (1923, 1925) in sheep and goats suffering from agalactia, a syndrome which includes mastitis, arthritis, keratitis, and vesiculo-pustular skin lesions. Since 1935, when Klienberger isolated another strain from cultures of rats infected with *Streptobacillus moniliformis*, a number of organisms of the same group, usually referred to as pleuropneumonia-like or "L" organisms, have been described as causing arthritis, abscesses, pneumonia, "rolling disease," and other complaints, chiefly in rats and mice. Free-living strains have also been isolated from sewage (Laidlaw and Elford, 1936), soil, and manure (Seifert, 1937).

In recent years organisms of the group have been identified in the urogenital tracts of both men and women. Dienes and Edsall (1937) isolated from a Bartholin abscess of a laboratory worker a strain which was possibly human, but at the time the infection was considered to be due to the patient's contact with rats. However, Dienes (1940) was really the first to record the presence of pleuropneumonia-like organisms (indistinguishable initially from the strains isolated from rats and mice) in the cervical secretions of females, the majority of whom were suffering from pelvic infections. The organisms were demonstrated in a third of the cases examined; in two cases they were associated with the gonococcus, and in one they persisted after the elimination of gonococci by sulphonamide therapy. Smith (1942) isolated six strains, five from the cervix and one from the urethral discharge of a male with non-gonococcal urethritis and arthritis. Dienes and Smith (1942) investigated 129 unselected cases and recovered pleuropneumonia-like organisms from twenty-three of seventy-seven cervical secretions, one of eight vaginal secretions, three of thirty-six prostatic secretions, and one of eight urethral discharges in males. All the four male cases in which cultures were positive were suffering from non-gonococcal prostatitis, two of these being complicated by acute polyarthritis and one by rheumatoid arthritis; cultures of the synovial fluid in two yielded negative results. A cervicitis was present in twelve of the females with positive cultures (seven associated with the gonococcus), and four were complicated by arthritis. The husband of one of the latter cases was also suffering from a non-gonococcal urethritis with polyarthritis, which the authors suggested was evidence of the transfer of the organism by sexual intercourse. Another case (not included in the above series), of a man with chronic prostatitis and tenosynovitis whose wife developed cervicitis and acute arthritis fourteen days after marriage (pleuropneumonia-like organisms being grown from the prostatic and cervical secretions), provided further evidence of transmission during sexual intercourse.

Dienes (1940), Brown and Hayes (1942), and

Salaman (1946) all demonstrated colonies resembling those of " L " organisms from what were considered to be pure cultures of gonococci. Brown and Hayes's discovery resulted from the examination of several pure strains of gonococci for associated organisms of the pleuropneumonia group. Using the bacteriostatic action of the sulphonamides on the gonococcus they inoculated serum agar plates incorporating sodium sulphadiazine with " cut out " sections containing the gonococcal colonies. While no gonococcal colonies developed along the path of inoculation, " L " forms were obtained in pure culture.

Salaman, using penicillin instead of sodium sulphadiazine, demonstrated the presence of vesicles containing minute granules morphologically indistinguishable from those of " L " organisms, in gonococcal cultures, but was unable to subculture the apparently pure " L "-like colonies growing at the edge of the penicillin zone. Salaman examined eighty strains of gonococci and saw what he considered to be " L "-like forms in all—in some after as many as fifty passages in culture.

Dienes (1947) recently isolated " L " organisms in cultures of *H. influenzæ*. He states that *H. influenzæ* is the fourth species of Gram-negative bacilli from the cultures of which " L " organisms have been isolated.

Beveridge (1943) demonstrated the presence of pleuropneumonia-like organisms in four of twenty-four cases of non-gonococcal urethritis in the male, while Harkness (1945-6), in an address to the Medical Society for the Study of Venereal Diseases in May, 1944, (partly concerning the ætiology of Reiter's disease), reported the frequent finding of inclusion bodies, possibly due to a virus, in the urethral discharge of primary uncomplicated non-gonococcal urethritis. He recorded one such case in which the complete syndrome of Reiter's disease subsequently developed : inclusion bodies and free elementary bodies were found also in conjunctival scrapings and lesions of keratodermia blennorrhagica, but no growth of organisms was obtained. At the time of these investigations the previous work on human strains of " L " organisms in the urogenital tract was not known, but as the elementary bodies were accompanied by rings, suggesting that the rings were developing from the granules, it was considered that the bodies were possibly a phase in the life cycle of " L " organisms. Johnston and McEwin (1945) reported two cases of non-gonococcal urethritis in which " L " organisms were isolated and inclusion bodies (cocco-bacillary basophilic elementary bodies) were demonstrated in the cytoplasm of epithelial cells in the urethral discharge. Klienberger-Nobel (1945) cultured " L " organisms from eighteen of forty-five cases of vaginal discharge attending a venereal clinic (the organism being isolated more frequently in recent cases of gonorrhœa and syphilis) and from twelve of thirty-six cases attending a gynæcological department (the organisms being relatively more frequent in cases with offensive vaginal discharges) ; " L " organisms were only detected in seven of fifty women attending an ante-natal clinic, and five of those with positive findings had or were still suffering from inflammatory complications of pregnancy.

Williams (1946) examined nineteen cases of non-gonococcal urethritis in Australian soldiers who had contracted the disease from native women in Borneo. In ten of these, inclusion bodies were present in urethral scrapings, and he tentatively suggested that they represented an intracellular phase of " L " organisms. Salaman (1946) isolated these organisms from 34 per cent. of men and 61 per cent. of women suffering from gonorrhœa, but from only three of forty-five men (7 per cent.) with non-gonococcal urethritis, three of thirty-eight (8 per cent.) from prostato-vesicular secretions, and two of thirty-five (6 per cent.) men with residual non-gonococcal urethritis following successful sulphonamide or penicillin therapy for acute gonorrhœa. " L " organisms were also isolated from the urine or urethra in four of twenty-six (15 per cent.) normal men, and from the cervical secretions in one of seventeen (6 per cent.) normal women. Beveridge, Campbell, and Lind (1946) isolated " L " organisms from fourteen of seventy (20 per cent.) cases of non-gonococcal urethritis in the male and from three of eleven (27 per cent.) females (all with cervical erosions) from whom men had contracted the disease. Seventeen positive results were also obtained from cervical and vaginal swabs of 101 apparently normal women attending a gynæcological clinic. No positive results were obtained with urethral washings from sixty-seven normal male medical students. These authors carried out complement fixation tests on the blood serum using an antigen prepared from two strains of " L " organisms isolated from cases of non-gonococcal urethritis, but there was no clear correlation with any clinical condition.

The Present Investigation

The purpose of this investigation has been an attempt to establish the role of " L " organisms in urogenital infections of males and females. Non-gonococcal urethritis, as is well known, has a very varied ætiology, and we have endeavoured to correlate the findings of " L " organisms on culture with the different types of urethritis.

We have examined a series of 206 male cases of

urethritis with non-gonococcal ætiology (132 abacterial and 74 bacterial), 47 cases of arthritis in association with non-gonococcal urethritis (41 abacterial and 6 bacterial), 46 females with non-gonococcal cervicitis or vaginitis, 16 cases of abacterial pyuria with no urethritis, and 157 cases of acute gonorrhœa (139 male and 18 female).

Cultures for " L " organisms were carried out on sixty-five normal individuals, and whenever possible on contacts of cases giving positive cultures. Cases with initially positive cultures were also re-examined after clinical cure.

All patients were examined for " L " organisms by smear and culture methods. Agglutination, complement fixation, and skin sensitivity tests were also carried out where possible.

Clinical Classification.—Our classification of the cases is given in Tables I and II.

Primary abacterial urethritis of venereal origin may be ushered in with signs and symptoms of either a subacute or an acute inflammation. Waelsch in 1901 was the first to describe the former, and in his original communication he stated that the disease was characterized by a long incubation period (in our experience from five to thirty days) mild subjective and objective symptoms, chronicity from the beginning, and poor response to treatment. There is also a typical urethroscopic picture (first described by Glingar in 1914), although Waelsch did not regard it as characteristic of the disease. Urethroscopy was carried out on all our cases, and none are included in this category which did not show the presence of the small greyish-white semi-transparent superficial and wedge-shaped excrescences. (The base of each is attached to the mucous membrane of the anterior urethra with no obvious involvement of the sub-epithelial connective tissue. The thin end of the wedge, which may be either pointed or blunted, lies free and protrudes into the urethra.) Urethroscopy carried out early will show large numbers of these bodies, especially in the roof and lateral walls ; but later in the disease the lesions, though flatter and having a cobble-stone appearance which has been likened to the nodules of trachoma, may be widespread or scanty, and a thorough search may be necessary for their recognition.

TABLE I

CLASSIFICATION OF CASES OF URETHRITIS

Condition		No. of cases	Pleuropneumonia-like organisms recovered	Per cent positive
Non-gonococcal urethritis	Bacterial	Venereal 38*	2	5
		Non-venereal 36†	—	—
	Abacterial	132‡	27	20
Non-gonococcal urethritis in association with arthritis	Bacterial	6	—	—
	Abacterial	41§	7	17
Non-gonococcal cervicitis or vaginitis		46	12	26
Abacterial pyuria with no urethritis..		16‖ (13 males ; 3 females)	2 (1 male ; 1 female)	12 (8 males ; 33 females)
Acute gonorrhœa		157 (139 males ; 18 females)	15 (12 males ; 3 females)	10 (9 males 17 females)
Normal individuals		65 (50 males ; 15 females)	—	—

* The 38 cases of bacterial urethritis of venereal origin include : desquamative urethritis (9) ; residual bacterial urethritis following successful penicillin therapy for gonorrhœa (2 with urethral stricture) (5) ; intrameatal condylomata acuminata (3) ; intrameatal chancroid (1) ; intrameatal ulcer (unknown ætiology) (1). The two cases in which pleuropneumonia-like organisms were cultured were considered to be primary mixed infections of these organisms and higher bacterial forms.

† The 36 cases of bacterial urethritis of non-venereal origin consisted of : traumatic urethritis (6) ; urethritis descending from urethral stricture, prostate, or upper urinary tract (30).

‡ Cases of abacterial urethritis are represented in Table II.

§ Early lesions of keratodermia blennorrhagica (2 cases cultured : negative) ; Synovial fluid (3 cases cultured : negative) ; residual urethritis after successful therapy for gonorrhœa (3 cases cultured, 1 positive).

‖ All cases were examined on three occasions for *M. tuberculosis*.

The acute type of abacterial urethritis of venereal origin may be indistinguishable clinically from acute gonorrhœa. The disease is characterized by a short incubation period (one to three days), an acute onset with profuse purulent or mucopurulent urethral discharge, and pain on micturition. Urethroscopy shows a red and inflamed mucous membrane with no infiltrations, and, as Hecht stated in 1927, the condition usually reacts rapidly to internal medication with sandal-wood oil.

The 41 cases of abacterial urethritis complicated by acute arthritis have been considered separately in spite of the fact that a large percentage had the urethroscopic picture of Waelsch urethritis; Reiter's syndrome was complete in 7 cases and incomplete in 34: 3 of the cases classified as abacterial were considered to be primary mixed infections of gonorrhœa and non-gonococcal urethritis. Two had received penicillin for acute gonorrhœa before admission under our care and developed acute polyarthritis, one two days and the other five days later. Gonococci, present in the urethral discharge of the third (in which Reiter's syndrome was complete), disappeared one hour after the first injection of penicillin, but 4·8 mega units which were given for a concomitant sero-negative primary syphilis had no beneficial effects on polyarthritis and skin lesions.

We observed seventy-four cases of urethritis which we considered to be due to micro-organisms other than the gonococcus. In many of the cases of venereal origin, organisms were seen in smears after a prolonged search; and cultures taken after cleansing the fossa navicularis with spirit yielded growths of staphylococci, diphtheroids, or micrococci (these may have been the normal saprophytic flora and due to inadequate cleansing before the taking of specimens). Details concerning the thirty-eight cases of venereal origin are given in Table I (footnote *). The urethral discharge in the

TABLE II

CLASSIFICATION OF CASES OF ABACTERIAL URETHRITIS

Condition	No. of cases	Pleuro-pneumonia-like organisms recovered	Per cent positive
Sub-acute urethritis (Waelsch)	57	21	38
Acute urethritis (simulating acute gonorrhœa)	10	5	50
Residual abacterial urethritis following successful penicillin therapy for gonorrhœa *N.B.*—Cases complicated by arthritis are considered separately (see footnote § to Table I.)	35	1	3
Traumatic (chemical) urethritis	11	—	—
Urethrorrhœa (excess of mucus, epithelial cells, occasional leucocyte)	5	—	—
Spermatorrhœa (spermatozoa, epithelial cells, occasional leucocyte)	3	—	—
Intrameatal condylomata acuminata	3	—	—
Trichomonas vaginalis	3	—	—
Intraurethral lymphogranuloma inguinale	1	—	—
Intraurethral herpes simplex	1	—	—
Intraurethral herpes zoster	1	—	—
Intrameatal chancroid	1	—	—
Intraurethral secondary syphilitic papular eruption	1	—	—
Renal tuberculosis (subsequently diagnosed and not included in this series)	1	—	—

nine cases of desquamative urethritis, often milky in colour, consisted of many epithelial cells containing large numbers of organisms, a few pus cells, and—in two—*T. vaginalis*. This condition repays immediate urethroscopy, as in our series five were suffering from urethral stricture, and a large patch of leucoplakia on the floor of the bulbous urethra was present in another.

Laboratory Investigations.—The following laboratory investigations were carried out on all cases. Smears were made of the urethral discharge after thorough cleansing with spirit of the external urinary meatus and fossa navicularis. One of these was examined direct and the other after staining by Gram's method.

Urethral scrapings were then taken with a platinum loop which was passed 2 inches down the urethra. Smears prepared from these scrapings were fixed immediately in acetone-free methyl alcohol and stained in 1/10 Giemsa for twenty-four hours. After inoculating a tube of nutrient or blood agar with a loopful of the discharge, 2 to 3 c.cm. of broth was squirted, by means of a pipette with a bulbous end, into the anterior urethra. The fluid was collected in a sterile dish and then injected into a 1 oz. screw-capped bottle of selective medium.

The basis of the selective media employed throughout this investigation was a papain digest of ox heart with the addition of 20 per cent. horse serum and 10 per cent. yeast extract. For the preliminary isolation of organisms of the pleuropneumonia-like group a sloppy agar was prepared by the addition of 0·3 per cent. agar, 1/2000 thallium acetate, and 100 units of penicillin per c.cm. of medium, the *p*H being carefully adjusted to 8 (Edward, 1947). When the optimum inoculum was once established, this medium would generally support only the growth of organisms of the pleuropneumonia-like group.

Following inoculation the sloppy agars were incubated ærobically at 37° C. for two or three days. Growth was generally visible to the naked eye in the form of small flocculent colonies after thirty-six to forty-eight hours' incubation, usually maximal in the mid and lower zones of the medium. Two or three drops of the primary growth were then subcultured on solid media (2 per cent. agar) prepared from the same base with the addition of 1 : 8000 thallium acetate. Plates were incubated ærobically in a moist chamber at 37° C. for two days. Plates were examined with the aid of a plate microscope for the identification of the characteristic colonies.

Culture Results.—The results are shown in Tables I and II. Cultures for "L" organisms were positive in thirty-six (14 per cent.) of 253 cases of non-gonococcal urethritis (including the cases associated with arthritis) in the male. When, however, the cases were subdivided on the basis of clinical and laboratory findings (Table II), five of ten (50 per cent.) cases of acute abacterial urethritis, and twenty-one of fifty-seven (38 per cent.) of subacute or Waelsch urethritis gave positive cultures.

It is interesting to note that two of the twenty-one positive patients with Waelsch urethritis contracted the disease by sodomy, and both had been previously under our care with gonorrhœa, one twelve months, and the other eight months previously, when cultures on the day following successful penicillin therapy yielded no growths of "L" organisms. In a further case, of a male aged 16 with non-gonococcal urethritis, bilateral conjunctivitis, polyarthritis, and balanitis, who also contracted the disease by sodomy, urethral washings yielded negative cultures. Laidlaw and Elford (1936) and Beveridge (1943) failed to isolate "L" organisms from fæces. We have recently examined anal swabs from nine passive homosexuals, and in one of these (the contact of one of the two cases referred to above) "L" organisms were isolated.

There were seven (17 per cent.) positives in forty-one cases of polyarthritis associated with abacterial urethritis. Cultures were negative from the synovial fluid and urethral washings from three cases, but of the seven cases in which Reiter's syndrome was complete urethral washings were positive in two and negative in five. Not irrelevant in this connexion is a further case of Reiter's syndrome (Harkness, 1945) in which "L" organisms were not cultivated during the course of the disease although positive growths were obtained two years after apparent cure. Re-examination showed the persistence of subacute urethritis. In two cases of Reiter's disease we were fortunate in observing the early vesicular lesions of keratodermia blennorrhagica. Cultures both of aspirated fluid and of scrapings from the lesions yielded negative results on ordinary laboratory and selective media; scrapings stained with Giemsa showed, however, the presence of elementary bodies in the cytoplasm of epithelial cells.

The two positive cultures in bacterial (non-gonococcal) urethritis of venereal origin were presumed to be primary mixed infections of the disease in its bacterial and abacterial forms.

Cultures were positive in twelve (26 per cent.) of forty-six females suffering from non-gonococcal cervicitis or vaginitis, while those carried out on 157 cases of acute gonorrhœa yielded "L" organisms in twelve (9 per cent.) of 139 males and in three (17 per cent.) of eighteen females. These figures may be compared with those of Salaman (1946), who reported the isolation of "L" organisms in eleven (61 per cent.) of eighteen females and twelve (34 per cent.) of thirty-five males suffering from gonorrhœa.

"L" organisms were isolated in one (3 per cent.) out of thirty-five cases with residual abacterial urethritis after successful sulphonamide or penicillin therapy for acute uncomplicated gonorrhœa: they

were found once in three similar cases in which acute polyarthritis had developed. However, it must be noted that culture examinations, in the majority of these cases, were carried out twenty-four hours after completion of treatment for gonorrhœa, and subsequent observation showed that the slight residual urethral secretion, due probably to resolution processes in gonococcal lesions, did not persist. Negative results were obtained from urethral washings of fifty normal males and from cervical and vaginal swabs of fifteen normal females.

Follow-up.—In twelve of our cases of Waelsch urethritis and one case of acute urethritis, culture examinations were carried out repeatedly both during and following illness. On completion of treatment, urethral washings in eleven cases of Waelsch urethritis with positive cultural findings and in the one case of acute urethritis yielded negative results, cultures being performed on three or more occasions in all. In the twelfth case cultures were negative on three occasions during a three months' observation period, but subsequently became positive, and at the same time urethroscopy showed a recurrence of excrescences: the disease may have remained latent until activated by some additional factor, or alternatively there may have been a reinfection. After further treatment, consisting of urethro-vesical irrigations with oxycyanide of mercury, 1 : 4000, and dilatations, the urine became clear with no threads and urethroscopy revealed a normal anterior urethra. Urethral washings were negative for " L " organisms on completion of treatment and during a four months' observation period.

Contacts.—Examinations were carried out on eleven contacts, seven females and four males, and cultures for " L " organisms were positive in three (27 per cent:) cases. All three occurred in the seven females from whom men were presumed to have contracted the disease (43 per cent.). Elementary bodies lying free and in the cytoplasm of epithelial cells from cervical scrapings were seen also in two cases with negative cultures. Two of the positive cultures were in young women recently married who had long-standing histories of vaginal discharge (urethritis of Waelsch type being noted in the husbands ten and fourteen days after marriage), one with vaginitis only (no *T. vaginalis* seen in wet specimens), and the other with cervicitis (no cervical erosion). The third was the wife (with mild cervicitis and no erosion) of a man referred to us for sterility and unaware of the presence of mild abacterial urethritis (Waelsch type). No growths were obtained in the four males, the wife of one being a positive case in the abacterial pyuria group. Three of the four males examined had recently been cured of gonorrhœa contracted from other women; and the fourth, although free from urethritis, was suffering from penile condylomata acuminata.

As already mentioned, we examined also the contact of one of our cases of Waelsch urethritis in which the disease was contracted by sodomy, and " L " organisms were isolated from anal swabs.

Smear Examinations.—Giemsa-stained smears were examined from all cases, and in many of those in which cultures were positive for " L " organisms epithelial cells were seen with the cytoplasm packed with large numbers of small bluish-staining bodies which showed marked pleomorphism: spherules, ovoids, rickettsia-like forms, and elementary-like bodies being observed, with a high proportion of ring-like forms showing wide morphological variation. These bodies were also noted lying free, both dispersed and in small clusters and showing a faint pale blue-staining matrix not unlike the Koch's bodies seen in infections with *Theilera parva*.

There was a distinctly higher percentage of positive smears than positive cultures, the former being more frequent in certain types, viz., acute urethritis and Waelsch urethritis.

It is considered by one of us (A.H.H.) that some of the inclusions may be due to a virus. This aspect will be considered in a subsequent communication.

Serological Methods.—Agglutinable suspensions and complement fixations antigens were prepared in the following manner. Flasks containing 250 c.cm. of papain digest-horse serum-yeast broth were inoculated with 2 c.cm. of four-day sloppy agar culture which had previously been carried through at least six subcultures in the same medium and were incubated anærobically at 37° C. for ten to fourteen days.

The culture was then centrifuged at 3,000 r.p.m. for one hour and the deposit washed thrice in normal saline. After the third washing the deposit was resuspended in a minimal amount of normal saline and thoroughly ground in a Griffiths tube. The volume was then made up with normal saline to 25 c.cm. and sprayed by the method described by Van den Ende and Edward (1942). The smooth homogeneous suspensions obtained in this way were standardized to an arbitary opacity using an absorptiometer. Phenol to 0·5 per cent. was then added in the case of agglutinable suspensions, and methiolate 1/1000 for the complement fixing antigens. Polyvalent suspensions were employed throughout in both tests and were prepared in the above manner from four strains isolated from different types of cases.

Agglutinations were carried out by the technique described by Wright (1912). Equal amounts of the standardized polyvalent suspension at a dilution of 1 : 2 and serial doubling dilutions of patient's serum were mixed, and, with the use of a throttled pipette, unit volumes of each mixture were successfully drawn up, each volume being separated by a bubble of air of

approximately equal size. Pipettes were sealed with plasticine, left to stand overnight at room temperature, and read with a hand lens the following morning. Besides being economical in materials, this method gave the clearest end point.

Specific hyperimmune sera prepared in rabbits agglutinated the polyvalent suspension, under the experimental conditions described, to a titre of between 1/128 and 1/256. Anti-horse serum sera similarly prepared in rabbits failed to agglutinate the suspension under similar conditions.

Complement fixation tests were carried out with the standardized suspension at a dilution of 1 : 5 and employing a 2 M.H.D. of complement. Sera were placed in a water bath for half an hour at 56° C. and subsequently diluted to 1 : 5 with normal saline.

0·2 c.cm. quantities of serum dilution, 1 : 5 antigen and complement, were incubated in the water bath at 37° C. for one hour. A similar volume of a 3 per cent. suspension of sheep cells sensitized with 5 M.H.D. of hæmolysin was then added and the tubes were reincubated for a further half hour.

Hyperimmune rabbit sera gave complete fixation with from 4 to 6 M.H.D. of complement.

Serological Investigations.—Twenty-five cases were examined by serological methods, samples of serum being taken at different stages during the course of infection. Cultures for " L " organisms were positive in fifteen and negative in ten, the urethritis in the latter being clinically indistinguishable from the former. The sera of twenty-five normal individuals were also similarly tested.

Both agglutinations and complement fixation tests were negative in all cases.

Skin Sensitivity Tests.—Intradermal tests were carried out in normal subjects. The phenolized polyvalent suspension was used, and it was determined that at a dilution of 1 : 20 this antigen gave rise to no more reaction in the normal skin than the control inoculation of 0·5 per cent. phenol saline. In sensitized guinea-pigs the intradermal inoculation of 0·1 c.cm. of the diluted antigen resulted after from five to seven hours in the development of a wide area of erythema with marked induration of the skin. Both control guinea-pigs and rabbits failed to react to the antigen.

This antigen is now being employed for skin testing in all our cases of non-gonococcal urethritis, but up to the present time no satisfactory reactions have been observed.

Storm-Mathisen (1946) described a skin test for Reiter's disease which employed a mixture of joint exudate and gland emulsion obtained from a case of this disease. A red papule (12 mm.), increasing slightly in size up to the eighth day and persisting for several months, developed forty-eight hours after intradermal inoculation in 5 cases diagnosed as suffering from Reiter's disease. A negative result followed the inoculation of eleven controls, including three with polyarthritis rheumatica.

Pathogenicity in Laboratory Animals.—Strains have been tested for pathogenicity in white mice, rabbits, and guinea-pigs. No lesions have been observed following inoculation by various routes.

In a single experiment thirteen-day old chick embryos were inoculated with sloppy agar cultures by the amniotic route. Embryos were harvested on the seventeenth day and immediately placed in 10 per cent. formol saline. Sections through the thorax, stained with hæmatoxylin and eosin and with Giemsa, showed a well-marked interstitial pneumonia in each case, and " L " organisms were noted in the Giemsa-stained sections in the œdema fluid filling the parabronchi and air capillary tubules. The experiment was later repeated with broth cultures, with similar results. Control embryos inoculated with sterile broth showed no lesions.

Discussion

According to Dienes (1940) " L " organisms may represent another variety of the many unknown saprophytic organisms : there is also the possibility advanced by Dienes and Smith (1942) that they may represent variant forms of bacteria, possibly gonococci. Beveridge (1943) suggested that they may be (1) saprophytes, (2) directly responsible for the disease, (3) saprophytes in the female, but that under certain conditions they may be transferred to the male during sexual intercourse and may cause urethritis. Johnston and McEwin (1945) suggest that their two cases of non-gonococcal urethritis may have been due to " L " organisms, a mixed infection with " L " organisms and a virus, or a virus *per se*. Beveridge and his collaborators (1946) went further in stating that " all the facts available are nevertheless consistent with the tentative hypothesis that the disease is, in the majority of cases, the result of infection by pleuropneumonia-like organisms." Salaman (1946), who, as already mentioned, cultured " L " organisms in only 7 per cent. of cases of non-gonococcal urethritis, suggests the possibility of three alternatives, (1) that gonococcal colonies are invariably contaminated by " L " organisms, (2) that " L " organisms and gonococci live in obligatory symbiosis, (3) that the " L "-like structures demonstrated in gonococcal colonies are a stage in the life cycle of the gonococcus. He does, however, consider the possibility that the vesicles seen may be degenerated gonococci, and we tend to favour this view.

On clinical grounds it appears that, in the present series, " L " organisms were isolated more frequently in certain types of non-gonococcal urethritis. They were present in pure culture in 50 per cent. of cases of acute primary abacterial urethritis and in 38 per cent. of cases of subacute primary abacterial urethritis (Waelsch), both conditions being venereal in origin. Based on an examination of smears we should place the percentage slightly higher. This difference may be accounted for by the difficulty of

cultivation of organisms of this group. In addition, investigations carried out on females presumed to have infected their sexual partners showed that 43 per cent. were harbouring " L " organisms.

The frequent association of " L " organisms in recent cases of acute gonorrhœa has been emphasized by Klienberger-Nobel (1945). Salaman in 1946 also reported them in eleven (61 per cent.) of eighteen females and twelve (34 per cent.) of thirty-five males, but we isolated them only in twelve (9 per cent.) of 139 males and in three (17 per cent.) of eighteen females suffering from acute gonorrhœa.

Klienberger-Nobel also stated that " L " organisms were frequently demonstrated in recent cases of syphilis. We isolated them in two such cases, both suffering from Waelsch urethritis which persisted in spite of penicillin therapy. Two further cases of Waelsch urethritis with positive cultures had penile lesions of molluscum contagiosum, one of which developed chickenpox three days after the onset of urethritis : at the same time this patient's wife had an attack of herpes zoster. The husband (with negative cultures) of the female positive in the abacterial pyuria group was suffering from penile warts.

Culture examinations of urethral washings from fifty normal men were all negative, which is in agreement with Beveridge and his collaborators (1946), who carried out culture investigations on sixty-seven medical students with completely negative results. On the other hand Salaman (1946) reported " L " organisms in four of twenty-eight normal men from a skin department of a military hospital, but admitted that one with positive findings showed a slight pyuria. Subacute urethritis, often symptomless, is easily overlooked and the evidence of this worker would have been more convincing if urethroscopy and smears after prostato-vesicular massage had been carried out on all cases yielding positive cultures and any with evidence of urethritis eliminated from his control group. One of our cases from which we isolated " L " organisms was referred to us on account of sterility and was at first placed in our control group. Careful examination, however, revealed the presence of a slight urethral discharge, threads in the urine, and the typical urethroscopic picture of Waelsch urethritis : cervical cultures from the wife of this patient were also positive for " L " organisms.

Similarly negative culture results were obtained in the examination of fifteen normal women admitted to the medical or surgical wards of a general hospital. All these control cases were normal on examination, and gave no past history of gynæcological abnormality. Klienberger-Nobel (1945) reported seven (14 per cent.) positive cultures for " L " organisms from fifty cases examined, Salaman one (6 per cent.) in seventeen, and Beveridge and his collaborators seventeen (17 per cent.) in 101. The above cases were regarded by the authors as normal, but it should be noted that those of Klienberger-Nobel were attending an antenatal clinic and those of Beveridge the gynæcological clinic of a public hospital.

Thus the high percentage of positive cultures for " L " organisms in cases of non-gonococcal urethritis, their absence from normal cases, their disappearance with successful treatment, and the recurrence of positive cultures with the recurrence of lesions all tend to support the conclusion that organisms of this group are responsible for certain types of abacterial urethritis of venereal origin.

" L " organisms were cultivated from urethral washings in seven (17 per cent.) of forty-one cases of arthritis complicating abacterial urethritis (so-called Reiter's disease). Only seven of these cases presented the complete syndrome of urethritis, conjunctivitis, arthritis, and keratodermia blennhorrhagica, and two (29 per cent.) gave positive cultures. So far we have failed to isolate organisms in the synovial fluid, conjunctival secretion, and skin lesions.

The effects of treatment of abacterial polyarthritis may throw some light on the ætiology of the disease. Organic gold salts (Findlay and others, 1940) and streptomycin (Powell and others, 1946) protect rats and mice from developing arthritis after injection in the pad with " L " organisms. Five of our cases received gold therapy (Harkness, 1945b and 1947), in one of which the result was equal to that obtained with fever. A further case received a course of 10 g. of streptomycin (0·25 g. six-hourly for ten days) which, after completion, showed an excellent result. (Bushby (1947) found that " L " organisms of human origin were sensitive to low concentrations of streptomycin.)

A growth of these organisms was obtained in two (12 per cent.) of sixteen cases of abacterial pyuria, the low percentage being possibly due to difficulties of isolation in urine. We have seen cases of this condition, and others have been reported in the literature (Baines, 1947) in which the initial lesion was an abacterial urethritis contracted by sexual intercourse.

Although evidence from cultures has favoured the view that " L " organisms are ætiologically related to certain types of abacterial urethritis, abacterial pyuria, and possibly Reiter's syndrome, serological tests have not supported this conclusion. Negative serological tests, however, cannot be regarded as strong evidence against the incrimination of " L " organisms in the ætiology of these diseases. For example, it is well known that complement-fixing

antibodies in certain gonococcal infections are difficult and sometimes impossible to demonstrate. Moreover, little is known of the antibody response to pleuropneumonia-like organisms in the human being. It may well be that antibodies are present in low concentrations and for such short periods that they are not demonstrable by the relatively crude methods at present available.

Summary

1. An attempt has been made to correlate the finding of pleuropneumonia-like or " L " organisms on culture with the different types of non-gonococcal urethritis.

2. Pleuropneumonia-like organisms have been cultured from twenty-one (38 per cent.) of fifty-seven cases of subacute abacterial urethritis (Waelsch), and from five (50 per cent.) of ten cases of acute abacterial urethritis. The clinical and urethroscopic pictures of both conditions are described.

3. Two cases of Waelsch urethritis in which cultures were positive for " L " organisms were contracted by sodomy, and anal swabs from the only contact examined were also positive.

4. Forty-one cases in which abacterial urethritis was associated with arthritis were examined, and " L " organisms were recovered from seven (17 per cent.) of cases. All cultures of joint fluid, blood, conjunctival secretion, or skin lesions were negative.

5. " L " organisms were recovered from two (3 per cent.) of seventy-four cases of bacterial urethritis (non-gonococcal), two (12 per cent.) of sixteen cases of abacterial pyuria, twelve (26 per cent.) of forty-six cases of non-gonococcal cervicitis or vaginitis, and fifteen (10 per cent.) of 157 cases of acute gonorrhœa (twelve (9 per cent.) of 139 males, and three (17 per cent.) of eighteen females).

6. Fifty normal males, and fifteen normal females were also examined by culture methods. In no cases were " L " organisms recovered.

7. Serological investigations—agglutination and complement fixation—carried out on all suitable cases at varying stages of the disease were invariably negative.

8. None of the strains isolated was pathogenic to laboratory animals.

9. Bodies morphologically resembling the pleuropneumonia-like organisms of mice and rats are described in smears prepared from urethral scrapings.

10. The ætiological relationship of " L " organisms to non-gonococcal urethritis, Reiter's syndrome, and abacterial pyuria is discussed.

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